Supplement

"Strain through the neck linker ensures processive runs: a DNA-kinesin hybrid-nanomachine study"

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Materials and methods

1. Sample preparation

DNA-kinesin from the main text were also used for the supplemental experiments. Long oligo DNA (13-40bp) were designed by modifying the sequences from Goodman et al. (2005) while considering the effect of the oligo DNA secondary structure (Yanagihara et al, 2007). To further explore the effects of the DNA-kinesin connection linker, other bi-functional linkers (AMAS: Pierce; KMUS: Dojindo) were used (see Figure S11C for details).

To further clarify the function of the neck linker, we also used protein-only based constructs (Figure S12C). These constructs were derivatives of the dimer construct K490 CLM. Three series of constructs were prepared. One had polyglycine inserted mutants in which 1-12 glycines were inserted between the neck linker end (L335) and the coiled-coil beginning (T336) (Hackney et al, 2003); another had part of the neck linker (I325-E334: IKNTVCVNVE) replaced with a random coil sequence (ESGAKQGEKG; Case et al, 2000); and the last had both. Note: for the latter two mutants, the replaced portion was slightly different from that used by Case et al (see Figure S12C and Supplemental Discussion for details). These protein-only based constructs were labeled with Cy5 at residue 215 or Qdot at residue 416.

2. Imaging

DNA-kinesin imaging was similar to that in the main text. In Figure S6A, S6B, S7A and S7B, we measured the displacement of the fluorescent spots and calculated the Mean Square Displacement (MSD). To determine the polarity of the axoneme, a very low concentration of Cy5-labeled wild type dimers (K490CLM 416) were mixed in

solution. From the kymograph images, the plus end of each axoneme was determined.

Protein-only constructs were labeled with Cy5 and imaged with TIRF microscopy. The images were recorded by an EMCCD (iXon DV860 DCS-BV; Andor). For some protein-only constructs, Qdot FIONA (Fluorescence In One Nanometer Accuracy) experiments were performed with a modified inverted type fluorescent microscope (IX-71, Olympus; Kikuchi, Mori and Tadakuma, in preparation). Qdots were excited with a green DPSS laser (532 nm; Compass 315M-100, Coherent). Images were taken by the EMCCD.

3. Data analysis

Images were analysed using ImageJ with custom-designed plug-in software. To measure the fluorescence intensity, an 8 pix diameter circle ROI was drawn on each frame for anti-correlation analysis (Figure S1B). A 10 x 10 rectangle ROI was drawn on the kymograph for fluorescence intensity distribution analysis (Figure S1C, S3B). The obtained fluorescence intensity for both methods was roughly the same (data not shown). Thus, we did not calibrate the data using the ROI area size. The precise conditions are written in the results section of the supplemental text.

To measure the motile probability of DNA-kinesin (Figure S5B), kymograph images were analyzed and the position of the fluorescent spots was determined by eye. The thresholds were the same as those in the main text (remained bound to the axoneme $\geq$ 5 frames (= 166 ms) and moving $\geq$ 2 pix (= 160 nm)). Since some spots showed a sudden disruption in the trace, molecules that jumped $\geq$ 1 pix within a single frame (33 ms) were removed from the motile probability analysis.

To calculate displacement, all molecules that remained bound to the axoneme $\geq$ 5

frames (= 167 ms) were analyzed, regardless of displacement or sudden disruptions in the trace.

For Mean Square Displacement (MSD) analysis, molecules that remained bound to the axoneme ≥ 60 frames (= 2 s) were used. A 1 x 10 pix ROI was drawn on the kymograph (corresponding to 33 ms (1 frame) x 800 nm (10 pix)), and the position was determined by the centroid algorithm using a built-in function in ImageJ. Data up to 30 frames (= 1 s) was fitted.

For Qdot FIONA, positions of the fluorescent spots were determined by a 2D Gaussian function fitting algorithm (Press et al, 1992; Tadakuma et al, 2006). With our system, position accuracy was as high as 1 nm at a frame rate of 1000 frame/s and typically bright spots, whose intensity corresponded to an accuracy of 2-4 nm, were used. Detection of the steps was judged by eye or by the automated step-finding algorithm in Kerssemakers et al. (2006).

1. Additional data of the DNA-kinesin structure (Figure S1-S3)

We analyzed the fluorescence intensity of observed spots to confirm whether these were of single molecules and whether DNA-kinesin assumed an expected structure.

First we checked nonspecific bindings of DNA to the axoneme. Cy5-labeled 20 nt oligo DNA (125 nM) was infused into the observation chamber. Nonspecifically bound spots were very low (0.18 spots / μm axoneme (= 7 spots / 40 μm total length of axoneme), data not shown). During observation, the concentration of DNA was about 1/100 of this condition (1nM), thus nonspecific binding was negligible. This is likely because the DNA backbone negative charge and the tubulin C-terminal negative charge repulsed.

Next we measured the fluorescence intensity of parallel-type (coiled-coil replacement type) DNA-kinesin (Figure 2 of the main text). Due to the technical limitations of synthetic DNA, TAMRA was used instead of Cy3. In parallel constructs, the distance between TAMRA and Cy5 is short, which allows FRET to be observed. However, when the two dyes are too close, it has been reported that a quenching effect is observed. To avoid this, we added a 13-nt polyT sequence to the 3' end of the TAMRA-labeled oligo DNA (Rasnik et al, 2006). In the presence of non-hydrolyzed AMP-PNP, DNA-kinesin bound tightly to the axoneme. Anti-correlative stepwise photo bleaching of the acceptor dye (Cy5) and simultaneous recovery of the intensity of the donor dye (TAMRA) was observed (Figure S1B). The fluorescence intensity of the acceptor fluorescent spots showed single-step photo bleaching and a single Gaussian function distribution, suggesting that the observed fluorescent spots corresponded to single molecules. In the presence of 1 mM ATP, motile spots were observed and the fluorescence intensity of those spots was similar to that of the AMP-PNP condition.

Thus we concluded that the motile spots were single DNA-kinesin dimer molecules.

Next we observed anti-parallel constructs. First, we observed a neck linker (C-terminal) labeled construct (K336CLM 333) hybridized with 10 bp DNA and checked whether the sense and anti-sense sequences correctly hybridized. Since the distance between the donor (Cy3) and acceptor dye (Cy5) was expected to be small (4.8 nm), a high FRET signal was expected to be observed meaning hybridized molecules were expected to be rarely observed in the Cy3 channel but clearly observed in the Cy5 channel. Indeed, with the sense (Cy3)-antisense (Cy5) combination, high FRET motile molecules were observed using 514 nm excitation. (Figure S2B). In contrast, sense (Cy3)-sense (Cy5) and antisense (Cy3)-antisense (Cy5) combinations showed no FRET signal (Figure S2C and D, respectively). To quantify these results, we defined the concordance rate P_{hybri} by equation s1,

$$P_{hybri} = N_{cy5} / (N_{cy5}+N_{cy3}) \quad \text{<Eq. s1>}$$

where N_{cy5}: number of Cy5 spots, N_{cy3}: number of Cy3 spots.

We considered two factors when making equation s1. 1) Hybridized 10-bp constructs were expected to show high FRET, thus hybridized molecules were expected to be observed only in the Cy5 channel and 2) direct excitation of Cy5 by the green laser (514 nm) was low (<5% intensity of that of the FRET signal, data not shown). Thus, non-hybridized molecules were expected to be observed only in the Cy3 channel. From these two conditions, we assumed that the total number of molecules excited by the green laser is (N_{cy5} + N_{cy3}). Leak fluorescence from the Cy3 to the Cy5 channel was less than 10%, indicating that the all molecules observed in the Cy5 channel were those from the FRET signal and so we defined the numerator as N_{cy5}.

Measurements were done at 514 nm, 2.7 mW, and 30 fps. To estimate the

hybridization efficiency under microscope, molecules that remained bound to the axoneme ≥ 2 frames (= 66 msec) were judged as microtubule (MT)-bound molecules and were analyzed without any regard for displacement. The dwell time of the DNA-kinesin monomer (K336CLM 333_10 bp) was 170 msec (data not shown). Therefore, under our ≥ 2 frames criteria, about 70% of the total bound molecules were expected to be counted. Since the affinity of the dimer and monomer to the MT might be different (e.g., higher MT affinity by the dimer than monomer), the obtained concordance rate under a microscope might be higher than that obtained by bulk measurements such as gel chromatography.

Results showed that 43% of the sense-antisense pairs ($N_{Cy3} = 405$, $N_{Cy5} = 303$) had FRET. On the other hand, a low ratio was measured for the sense-sense pair ($P_{hybri} \leq 1\%$; $N_{Cy3} = 681$, $N_{Cy5} = 1$) and the antisense-antisense pair ($P_{hybri} \leq 2\%$; $N_{Cy3} = 538$, $N_{Cy5} = 10$). Note: we could not consider exactly the effect of the molecules that hybridized with the same dye, Cy3 oligo - Cy3 oligo or Cy5-oligo - Cy5-oligo, since these showed no FRET signal. However, fluorescence intensity analysis suggests these probabilities are low (see below).

Next we measured constructs connected with 6 and 15 bp DNA. Because constructs with a 15 bp DNA connection showed lower FRET efficiency than 6 and 10 bp, a fluorescence signal was observed in both Cy3- and Cy5-channels simultaneously. Thus, all molecules excited by the green laser could be observed in the Cy3 channel, and so we redefined the concordance rate P_{hybri} to P_{hybri}' as in equation s2,

$$P_{hybri}' = N_{cy5} / N_{cy3} \quad \text{<Eq. s2>}$$

P_{hybri} and P_{hybri}' were 7% ($N_{Cy3} = 1419$ / $N_{Cy5} = 108$) and 71% ($N_{Cy3} = 731$ / $N_{Cy5} = 520$) for 6 and 15 bp DNA constructs, respectively. This was expected as a longer

complementary strand results in more stable hybridization.

Additionally, we measured the fluorescence intensity of many K336CLM 333 constructs with variable DNA length and calculated FRET efficiency (Figure S3). Fluorescent dyes were attached at both ends. Assuming DNA had a duplex structure, we found FRET efficiency lowered upon increasing the number of DNA nucleotides (Figure S3D). Some motile molecules showed much lower FRET efficiency than expected (0-20% FRET efficiency with 10-bp DNA constructs, 16% = 25/156 molecules). This may be because the Cy5 was photo bleached or in a dark state, or sense (Cy3)-sense (Cy3) hybridization occurred. However, based on the fluorescence intensity analysis, the latter possibility was dismissed. If sense-sense hybridization occurred, fluorescence intensity would be double that of a single Cy3 molecule. However, the intensity distribution showed that very bright molecules were of a very small proportion (in 10-bp DNA constructs, 4% = 6/156 molecules). Thus, fluorescence intensity analysis confirmed correct hybridization for most of the observed DNA-kinesin molecules.

Taken together with the results of Figure 1 in the main text, which confirmed this finding by use of a restriction enzyme, we concluded that most DNA-kinesin molecules hybridized correctly.

2. Additional data on DNA-kinesin activity (Figure S4-S7)

To evaluate precisely the effect of DNA length on motile properties, we measured the velocity, run length and residence time, the last being defined as the time from association to dissociation with MT, of a neck linker connected construct (K336CLM 333 and 328) with various DNA lengths (Figure S4). With longer DNA, the velocity decreased only slightly. In contrast, the run length and the residence time showed high dependency on DNA length. These results are similar to those of a bulk kinetic analysis study (Hackney et al, 2003), but very different from those of a recent single molecule experiment (Yildiz et al, 2008). Yildiz et al. measured polyproline inserted neck linker extended constructs, showing that run length was independent of the neck linker length whereas velocity was greatly dependent. We discuss in the main text the reasons for these contradicting results. Briefly, however, we speculate that these differences reflect a difference between the constructs despite the original wild type construct being from the same plasmid.

Figure S5A shows kymographs of mid connected constructs. The dependency of motile probability on DNA length for some constructs is also presented (Figure S5B). Constructs connected at position 23 and 101 showed diffusive movement, while constructs connected at 43 and 215 were stationary. The reason for this difference has not yet been clarified.

Figure S6A shows the displacement distribution of some connect positions at maximum motile probability conditions. The C-terminal (neck linker) and N-terminal connected constructs showed unidirectional movement toward the MT plus end. Mid connected constructs showed diffusional movement. To evaluate the mode of movement, mean square displacement (MSD) was calculated from the trajectory of molecules that

remained bound ≥ 2 s and fitted with equation S3,

$$MSD = at^b + c \quad \text{<Eq. S3>}$$

where t is time and a, b, c are constants

If the motile mode is simple active transport, b = 2; if simple diffusion, then b = 1. The obtained values were b = 2.0, 1.6 and 1.8 for connect positions 2, 7 and 328, respectively. On the other hand, the obtained values were b = 1.2 and 0.9 for connect positions 23 and 101, respectively. Because the value for the control wild type dimer construct (K490CLM 416) was b = 2.0, we concluded that constructs with N- or C-terminal connections were driven by active transport. Furthermore, since monomers were stationary, our data also showed a slight effect by dimerization (probability of molecules that move more than 1 pix was 0.0025 (= 1/400 molecules)). Further studies, such as FIONA, FRET and optical trap experiments, are needed to analyze the precise movement of our DNA-kinesin.

Figure S7A shows the displacement distribution of hetero-dimer constructs at maximum motile probability conditions. Results show forward bias in the displacement for all constructs except 215-337. These results are expected since one head (position 337) of the hetero-dimer could achieve full bias through the neck linker. To distinguish the difference between the connect positions, we measured the MSD (Figure S7B). The obtained values were b=1.9, 1.7, 1.5, 1.5 and 1.5 for hetero-dimers 324-337, 23-337, 43-337, 101-337, 215-337. Thus we concluded that hetero-dimers 324-337 and 23-337 move processively.

3. Detail structure of DNA-kinesin and its effect on activity (Figure S8-S11)

In this section we estimated the effect of the DNA-kinesin structure on the collision density of the detached head to the next binding site and on the internal strain between the leading and trailing heads.

3-1 Rough structural character of DNA-kinesin (Figure S8)

We connected two monomers with a rod-like short DNA. To reveal the unique character of DNA-kinesin, we compared the structure of DNA-kinesin to that of the protein-only based construct.

In native kinesin, the neck linker (324-336) is located at the C-terminal of the head (catalytic domain). The two heads are dimerized by the following C-terminal coiled-coil. Amino acids of the neck linker unbound to the head have been thought to assume a random-coil structure that acts as an entropy spring (Rice et al, 2003). Meanwhile the coiled-coil part is thought to act as a rigid rod. Its overall structure is like that shown in Figure S8A. Recently, to expand the detached head's reach, a random coil structure such as polyglycine (or glycine-serine repeats; persistence length, lp = 0.8 nm; Sahoo et al, 2006) or a relatively rigid structure such as polyproline (lp = 4.4 nm; Schuler et al, 2005) were inserted into the boundary of the neck linker and coiled-coil. Either of these structures can be treated as a spring.

In contrast, we used DNA rigid rods (lp = 50 nm; Bustamante et al, 1994) to connect the two heads in DNA-kinesin. Amino acids from the neck linker and carbon chains that connect the DNA and kinesin act as the spring. So the overall structure is like that shown in Figure S8B.

Next we considered the rigidity of DNA, which consists of three parameters:

extension, bending and torsion. The extension property of DNA has been explored by optical trap studies. From these experiments, DNA was thought to be very rigid, as the elastic modulus was ~1000 pN (Wang et al, 1997), although, recent experiments argue that DNA might be more flexible at longer lengths (Mathew-Fenn et al, 2008). Nevertheless, we assumed DNA to be a rigid rod, since highly motile DNA-kinesin in our experiments had very short DNA (-12 bp). For bending, we assumed that the DNA ends behave according to the Worm-Like Chain (WLC) model (Eq S4; Thirumalai and Ha 1988), which has been observed experimentally for polyproline residues (Schuler et al, 2005), as described by

$$P(r) = \frac{4\pi C r^2}{L^2[1-(r/L)^2]^{9/2}} \exp\left(-\frac{3L}{4l_p[1-(r/L)^2]}\right) \qquad \text{<Eq. S4>}$$

where r: distance between DNA ends,

L: DNA contour length,

P(r): the probability of a DNA with r,

lp: (bending) persistence length of DNA,

C: normalization factor

For simplicity, we considered a one-dimensional distribution. Assuming that lp = 50 nm, the differences in length from bending are less than 1% of the DNA mean end-to-end distance (6-40 bp). Note: in real experiments, DNA was connected to kinesin by a soft spring. Thus actual distributions are much wider than those calculated by Eq. S4 (see Figure S9C).

For torsion, the flexibility of the carbon chains that connect DNA and kinesin (see

Figure S9A) assure free rotations of the detached head around DNA because the carbon chains are connected to single c-c bonds, which act as swivels. Therefore, at the moment the detached head collides with the tubulin binding site, the effect of the direction of the head does not need to be considered; only the longitudinal rigidity of the heads does (Hayashi and Harada 2007; Strick et al, 1999)

Next, we compared the longitudinal distribution of the heads using protein-only constructs and DNA-kinesin constructs. We assumed a lp = 0.8 nm and 4.4 nm for polyglycine and polyproline, respectively, and examined the effect of the carbon chain. Figure S8C shows the one-dimensional probability ($P(r)/4\pi r^2$) distribution of one head with the other head fixed at the origin and a mean end-to-end distance between heads of 7 nm for a DNA-kinesin construct and neck linker extended kinesin. The polyglycine construct had a broad distribution with a maximum at the origin. The polyproline construct also had a peak at a specific distance, but the probability at the origin was not zero (data not shown). In contrast, for DNA-kinesin, the probability distribution had a narrow peak at the length of the DNA, thus it was virtually zero at the origin. Note: the probability distribution is much wider than that of DNA alone because the soft spring generated by the amino acids and carbon linkers are considered. The low stiffness of the linkers is the main reason for the width of the distribution. As DNA extends, the distribution moves in parallel since DNA can be treated as a rigid rod. However, as mentioned above, recent experiments showed that the longitudinal extension was unexpectedly softer than expected for a rigid rod (Mathew-Fenn et al, 2008). In that paper, the end-to-end distance of short DNA was measured with small-angle X-ray scattering interference. The mean end-to-end distances increased linearly with an increase in DNA bp. In contrast, the distribution of the end-to-end distance was much

wider than expected and increased quadratically with the number of base pairs. The distribution thus matched the predicted value obtained from the classical rod-like structure for up to 10 bp. However, the model did not hold for longer DNA. For example, at 35 bp DNA, the variance was about 10 times that obtained from the classical rod-like model. Thus, for longer DNA, more careful handling might be required. However, in this work, the high efficiency motile condition was restricted to DNA of up to 12 bp, so we treated DNA as a rigid rod.

3-2 Estimate of the collision frequency of the detached head (Figure S9-S10)

The DNA and kinesin connection is shown in Figure S9A. The DNA 3' terminal had an amine that was modified to maleimide with an amine-sulfhydryl-reactive linker, which had an aliphatic spacer composed of carbon chains. Then maleimide was attached to a thiol group on the kinesin head. The DNA-kinesin structure was such that the kinesin head connected to the rod through a spring (Figure S8). To simplify the situation, we assumed that the spring was only on one side (Figure S9B) and that the carbon chains and amino acids acted as an entropy spring based on the WLC model (Eq. S4). When calculating P(r), we assumed the unit length of the carbon chain to be 0.13 nm. The number of carbon chains (N_{carbon}) per head was calculated from equation S5. The unit length of an amino acid was assumed to be 0.34 nm. The number of amino acids per head was calculated from equation S6.

$$P(r) = \frac{4\pi C r^2}{L^2[1-(r/L)^2]^{9/2}} \exp\left(-\frac{3L}{4lp[1-(r/L)^2]}\right) \qquad \text{<Eq. S4; reprint>}$$

where L (contour length) = 0.13 x N_{carbon} + 0.34 x $N_{amino\ acid}$

$$N_{carbon} = (N_{amino\text{-}linker} + N_{maleimide\ modified\ reagents}) \times 2 \qquad \text{<Eq. S5>}$$

where

$N_{amino\text{-}linker} = 8$

$N_{maleimide\ modified\ reagents} = N_{spacer}$ + maleimide group (3 bonds)

N_{spacer} = 2 (AMAS), 6 (EMCS), 11 (KMUS)

$N_{amino\ acid}$ = (Connect position leading – base point) + (Connect position trailing – base point) <Eq. S6>

where base point is the first residue that is an unbound free amino acid in the neck linker

base point for C-terminal (neck linker) connection is

leading head (ADP state; undocked structure): 324

trailing head (ATP state: docked structure): 332

base point for N-terminal connection is

leading head (ADP state; undocked structure): 9

trailing head (ATP state: docked structure): 4

base point for mid connection is

$N_{amino\ acid} = 0$

In this paper, the persistence length of the linker was assumed to be lp = 0.8 nm. To be precise, we considered the carbon chain and amino acids separately. However, for simplification, we assumed the lp for both was the same. The reported value for glycine-serine repeats, neck linker and polyproline was 0.8, 1.4, 4.4 nm, respectively.

Next we calculated the collision probability. From equation s4, we calculated the distribution of one head with the other head fixed (Figure S9C upper), then normalized the distribution to the unit area (Figure S9C middle). After mirroring the distribution at

the origin (Fig.9C lower, black), the whole distribution shifted in parallel the length of the DNA (Figure S9C lower, red).

Using $P(r)/4\pi r^2$, we predicted the collision frequency of one head to the tubulin binding site. To calculate the collision frequency, we surmised the structure of the kinesin head. The structure of the neck linker in particular must be carefully considered, since it is well known that the neck linker takes a dramatic conformation change depending on the nucleotide state of the head. Previous reports (Sindelar et al, 2002; Kull et al, 1996; Turner et al, 2001) suggest there are at least three stable states, two of which are "docked" and "undocked" (Figure S10A). The docked state is when either ADP or AMP-PNP (a non-hydrolyzed ATP analog) is bound to the head. In this state, the neck linker is attached to the front part of the head. In the undocked state ADP is bound to head but the neck linker is unbound to the head and thus freely diffuses around the root of the neck linker. The third state, which also has ADP bound to the head and was first reported from the crystal structure (PDB entry 1II6) of kinesin-5 (Eg5), was also considered. In this state, the neck linker binds to the back part of the head towards the opposite direction of movement (Figure S10B; hereafter "back docked state"). Recently it was reported that another kinesin protein family, kif22 (KID), also takes a similar version of this structure (PDB: 3BFN).

In this study, we assumed that the head assumed a docked state (PDB: 1MKJ) when in the ATP state. In the ADP state, crystal structures of the docked and undocked states were obtained for conventional kinesin, suggesting a dynamic transition between the two. However, from the reported data of cryo-electron images (Rice et al, 1999) and EPR data (Rice et al, 2003; Sindelar et al, 2002), we assumed that the undocked state was predominant when ADP was bound. The crystal structure for conventional kinesin

in a nucleotide-free state (hereafter ϕ state) has not been reported. A low-resolution cryo-electron image of the ϕ state (PDB: 2P4N) showed the total structure resembles the undocked state rather than the docked state. However, because of the low resolution, the position of the neck linker was unclear. The crystal structure for the ϕ state has been reported (PDB: 1RY6) for another kinesin family member, KinI, which has a motor domain in the middle part of its polypeptides. However, the nucleotide pocket of the KinI head is not completely empty; instead, it is occupied by sulfate. Again, while the overall structure resembles the undocked state, the position of the neck linker is unclear. Finally, Kar3, which moves toward the minus MT end, has had its cryo-electron structure reported (Hirose et al, 2006). However, the structure of α4, which is important for microtubule binding, is significantly different. Taken together, we assumed that the neck linker takes the undocked state in the ϕ state. If kinesin assumes a back docked state in the ϕ state, the effective distance between the heads becomes shorter, and the internal strain between heads becomes weaker when using the same DNA length.

In Figure S10C-F, we present the results from connect position 328, which is located half way on the neck linker. In the docked state, there are no amino acids that assume a random coiled structure between the two connect positions for the trailing head and that only the carbon chain acts as an entropy spring. Furthermore, for the leading head, for which we assumed an undocked state, there were few free amino acids (4 amino acids: 324-328). Thus the effect of the amino acids chain is expected to be low, allowing for easy interpretation of the results. To calculate the collision frequency, one should assume the width of the tubulin-binding domain to which kinesin can bind. We assumed it to be 0.1 nm. Regardless, whether the width changes or not, the overall structure of the collision frequency does not change (data not shown).

3-3 Estimate of bias length upon docking the neck linker

Connecting to the N- or C-terminals (neck linker) alters the bias length of the power stroke (docking of neck linker to the head; Figure 5D). In this study, we assumed that the structure of the docked state is the same as that of crystal structures since all of our constructs have a full neck linker (324-336). To calculate the bias length, we assumed that the base point of the bias was 324 and that the bias length was equal to the distance between 324 and the labeled position. In the crystal structure 1MKJ, not all N-terminal residues attached to the neck linker through a hydrogen bond, but the latter half residues (4-8) do. Thus, for the construct connected at position 2, we used the distance between position 4 and 324 (1.9 nm) as the bias length (c.f. the distance between position 2 and 324 is 2.3 nm).

3-4 Estimate of internal strain between the two heads (Figure S11)

For an efficient processive run, our results showed that it is important to connect the heads through the C-terminal (neck linker). This may be because the C-terminal regulates the activity of both the leading and trailing head. It has been suggested that the neck linker acts as an entropy spring and that the internal strain between heads regulates the head's activity (Uemura and Ishiwata 2003; Yildiz et al, 2008). To evaluate the effect of internal strain, we calculated the internal stain and checked the correlation between internal strain and the experimental results. The internal strain depends on the length of the connected spring and the distance between the two connect positions. For distance, we used the same value found when calculating the collision frequency above. Since the obtained values of internal strain depend on the model, we do not discuss the

absolute value but only the relative one. We calculated the internal strain assuming the WLC model (see also Figure S11A).

$$f(r) = \frac{k_B T}{l_p}\left[\frac{1}{4(1-x/L)^2} + \frac{x}{L} - \frac{1}{4}\right] \quad \text{<Eq. S7>}$$

where, k_B: Boltzmann constant, T: temperature, lp: persistence length, D: distance between two connect positions, L: contour length, and $x = D - L_{DNA}$, where L_{DNA}: length of DNA (end-to-end distance).

Equation S7 shows that the estimated internal strain depends on the value of lp. Many values of lp have been reported, all depending on the composition of the amino acids: 0.8, 1.4, and 4.4 nm for glycine-serine repeats, neck linker, and polyproline, respectively. DNA-kinesin has an amino acid part and a carbon chain part, thus estimates should be carefully done. But in this paper, we assumed that lp is constant (0.8nm) for all constructs to simplify the qualitative evaluation.

In the main text and supplement, we showed that run length (motile probability) and velocity depend on the connect position and discuss the effect of bias length and internal strain. These results, though, were obtained by changing both the connect position and the DNA length. Thus, the results may, in fact, not depend on bias length or internal strain, but rather solely on the connect position. To clarify this, we took advantage of DNA-kinesin, whose distance between the heads and its spring character between heads can be independently changed by changing the DNA length and linker length, respectively. So we explored the effect of both parameters at a fixed position (Figure 7). We chose position 328, the halfway position of the neck linker to minimize the effect of free amino acids from the neck linker that do not bind to the head, and thus maximize the effect of the three different carbon chains: AMAS, EMCS and KMUS

(Figure S11C (same as Figure 7C)). The collision frequency was the same for all three (Figure S11D). This is expected since the end-to-end distance of the DNA mainly determines the collision frequency in DNA-kinesin. On the other hand, the estimated internal strain is different, and the internal strain ratio between AMAS and KMUS ranged between 1.5 and 3 (Supplementary Figure S11E). Using these conditions, we explored the effect of the carbon chain spacer in Figure 7 of the main text. Results confirm that the run length depends more on internal strain than velocity does (Figure 7E).

Discussion

1. Contribution of forward bias by the neck linker power stroke on motility (Figure S12)

To explain the high processivity and high speed of kinesin, two functions have been proposed. One is the power stroke, which shifts the detached head forward, while the other is communication (regulation) between the leading and trailing heads (Figure S12A).

To evaluate the contribution of these two functions, it is useful to compare the activity of different constructs. In the main text, we compared the following 4 conditions: 1) both the power stroke and C-terminal communication (hereafter Cterm Communication) are active, 2) only the power stroke is active, 3) only Cterm communication is active and 4) both mechanisms are inactive. Our results from the main text showed that both functions are crucial for processive movement. However, in the main text, condition 3 (* in Figure S12B) was investigated only for the construct connected at the root of the neck linker (position 324). Here, we consider other constructs that satisfy condition 3.

Previous results showed that protein-only constructs in which the neck linker was replaced with a random coil sequence do not assume a stable docked state (Case et al, 2000). Thus, these constructs correspond to condition 3. Furthermore, in the DNA-kinesin construct constructs in which the neck linker is truncated (e.g., K324CLM, see Figure S12D) and connected through the C-terminal also correspond to condition 3. Case et al. reported that the ATPase of the random-coil replaced construct was 1/3 of that of WT, but the gliding speed of the microtubule (MT) was 1/400. The decrease in gliding speed, however, might be the result of the short reach in the detached head. Thus,

we inserted polyglycine between the neck linker and the coiled-coil and observed neck linker extended constructs. Our neck linker extended mutants slightly differed from those in Case et al. They replaced amino acids 323-332 of the neck linker with a random-coil, whereas we replaced amino acids 325-334 and inserted a polyglycine between L335 and T336. We changed the replacement position for two reasons. First, the crystal structure of Eg5 (PDB: 1II6) showed that K323 (K357 in 1II6) creates a hydrogen bond with the conserved residue D49 (D69 in 1II6). Second, point mutation analysis of conventional kinesin (K490CLM) revealed that the construct with the F48A/D49A mutation could not attach to MT, and that the K323E mutation decreased the mean run length to $1/4^{th}$ its WT value (data not shown) while the velocity was unchanged. These results suggest that K323 interacts with D49 in conventional kinesin like Eg5. Thus we judged that K323 is important for processive movement and shifted the replaced position by two amino acids toward the C-terminal end.

Results from single molecule imaging have showed that the neck linker replaced mutant did not walk in the manner reported by Case et al. (2000), although inserting 2-9 glycines, which made the length of the linker 14-23 aa per head, made the motor processive, albeit with low probability (10-15%) and speed (10-25 nm/s) (data not shown). Following further neck linker extensions (insertion of 12 glycines making the linker 26 aa/head), no movement was observed. Peak speed (25 nm/s) was observed with a 5 glycines insertion construct (linker length 19 aa/head), suggesting that there is an optimal distance for processive movement. On the other hand, DNA-kinesin constructs with a truncated neck linker (Figure S12D) showed no movement (data not shown).

Using FRET experiments, Case et al. showed that the replaced neck linker did not

stably attach to the head even in the docked condition. Taken together with our above results, one might assume that the neck linker is not critical for processive movement, which appears inconsistent with the conclusion of the main text (Figure 6 in main text). However, we speculate that the replaced neck linker (random coil sequence) might temporarily attach to the head, allowing the random coil sequence to act as an effective neck linker. Indeed, biochemical experiments showed truncating the neck linker decreased ATPase to 1/10 that of WT (Okada et al, 2003; Nitta et al, 2008), but replacing the neck linker only slightly affected ATPase (1/3 - twice that of WT; Case et al, 2000; Nitta et al, 2008). Furthermore, the neck linker attaches to the head by a β-sheet structure in which residues interact with the main chain, suggesting that even if the replaced residues prefer a random-coil structure than a β-sheet structure, it is possible that there is a temporal interaction between the side-chain residues and the head. Optical trap experiments showed that the rising time of the 8-nm step is about 100 μs (Nishiyama et al, 2001; Carter and Cross 2005). Thus docking for less than 1 ms might be sufficient for an effective neck linker, even if the replaced residues do not attach stably to the head. Furthermore, a simulation study showed that full docking is not needed to attach the detached head to the next binding site (Czovek et al, 2008). To confirm this, one should do high temporal resolution experiments such as EPR to observe the docking state of the neck linker. However, this is challenging. Because our data showed that only 4 aa (1.5 nm = 4 aa x 0.38 nm, data from DNA-kinesin with a connect position at 328; 4 aa = 324-328) is enough for processive movement, the detection technique requires both high temporal and spatial resolution.

From the results of the main text and this section, we concluded that the dual function of the neck linker (power stroke and communication) is crucial for processive

movement.

2. Mechanism of internal strain (Figure S13)

2-1. Internal strain hypothesis

Native kinesin walk processively more than 100 steps after encountering a MT. For this high processivity, it is important to keep the heads coordinated such that at least one is always attached to the MT. Internal strain is thought to be key for the head-head coordination mechanism driving processivity.

Internal strain occurs between two heads. Thus, its effects are apparent in the two-head bound state, but not in the one-head bound state. There are two possible effects: 1) internal strain contributes to the transition process from the two- to one-head bound state or 2) it contributes to the transition process from the one- to two-head bound state.

Contributing to the transition process from the two- to one-head bound state, internal strain can 1-1) accelerate detachment of the trailing head (Figure S13A) or can 1-2) inhibit ATP binding to the leading head (Figure S13B). If it promotes the one- to two-head bound state transition, internal strain can 2-1) inhibit the rebinding of the detached head to its original position (Figure S13C) or can 2-2) accelerate forward binding (Figure S13D). One of these mechanisms might be more important than the others or a combination of mechanisms might be critical for kinesin walking. We discuss each mechanism below.

1-1) Accelerating detachment of the trailing head (referred to as the “mechanical gate model” in the main text; Figure S13A)

Yildiz et al. (2008) proposed this model. They inserted polyproline residues or glycine-serine repeats into the boundary of the neck linker and coiled-coil, and measured the motility of these constructs at the single molecule level. They found that by increasing the number of inserted proline residues, the velocity of the constructs decreased, but run length remained almost the same as wild type (WT). Furthermore, external force (what they refer to as "assist force") applied by the optical trap recovered the velocity. Because the distance between the two heads is constant in the two-head bound state, inserting more proline residues relaxes the spring, which decreases the internal strain. In addition, the assist force accelerates the trailing head's dissociation. From these results, they concluded that the reason for the decrease in velocity was lower acceleration in the trailing head's dissociation.

1-2) Inhibition of ATP binding to the leading head (referred to as the "chemical gate model" in the main text; Figure S13B)

When walking, kinesin alternately repeats one-head and two-head bound states to move in a 'hand-over-hand' fashion. One ATP is hydrolyzed for each step (Schnitzer and Block 1997; Hua et al, 1997). At saturating ATP concentration (1 mM), kinesin spends most of its time in the two-head bound state. Thus, the rate-limiting step is the hydrolysis of ATP. When ATP concentration is low, ATP binding becomes rate-limiting. At low ATP, kinesin waits for ATP in the one-head bound state (Hackney 1994; Hackney et al, 2003; Alonso et al, 2007; Mori et al, 2007). When ATP is added to the one-head bound state, ADP is released from the tethered head upon binding to tubulin such that it becomes the leading head (Hackney 1994; Hackney et al, 2003). ATP can only bind when the head is in the nucleotide-free state (ϕ state). Thus, at the end of the ATPase

cycle, when the trailing head detaches from tubulin, the original leading head (bound head in the one-head bound state) is in the ϕ state. Furthermore, when the formerly tethered head releases ADP upon reattachment to the next forward MT binding site such that it becomes the lead head, it too is in the ϕ state, and commences a new ATP cycle. Because the leading head is in the ϕ state when the ATP cycle begins and ends, it is reasonable to assume that the leading head is kept in the ϕ state throughout the cycle, at least at low ATP.

Optical trap experiments showed that ADP affinity for the head is dependent on load direction (Uemura and Ishiwata 2003). Thus an internal strain hypothesis has been proposed where internal strain ensures the inhibition of ATP binding to the leading head.

2-1) Inhibiting rebinding to the detached position (Figure S13C)

The trailing head of the two-head bound state hydrolyzes ATP and releases Pi to take the ADP state, which then triggers detachment such that kinesin takes the one-head bound state. If the detached head rebinds to its original position, then the rebound head might release ADP and take the ϕ state, meaning one ATP was used but no step was achieved, which is an inefficient use of energy. However, since the transition between one- and two-head bound states is slow (0.01-1 / s; Hackney et al, 1994, 2003; Kawaguchi et al, 2003; Mori et al, 2007), a mechanism to prevent rebinding of the detached head may exist. To prevent this inefficiency, the collision frequency of the detached head to the former binding site must be reduced. This can be accomplished by attaching it to the anchored head in a way that prevents attachment to the original position (Alonso et al., 2007). One other possibility is to prevent ADP release from the detached head if it rebinds to its original position. This can be achieved through internal

strain in the neck linker. This was seen when cross-linking the neck linker to the head fixed in the docking state, where ADP release was blocked (Hahlen et al, 2006).

2-2) Acceleration of forward binding (Figure S13D)

At saturated ATP concentration (1 mM), most detached heads bound to the next binding site correctly (95%, data not shown) with a low probability for side and back steps, despite both sites being within reach. Here too, internal strain is a factor, as it prevents ADP release when the head binds to an undesirable location.

2-2. Interpretation of DNA-kinesin results

Our DNA-kinesin results show that extending the distance between the heads by changing the length of the DNA caused the velocity to slow down and the run length to shorten. However, velocity was only slightly sensitive to DNA length compared to the effects on run length, residence time and motile probability (main Figure 4 and Supplementary Figure S4).

To determine the dominant mechanism, we first consider what happens if each of the four mechanisms are impaired.

1-1) Accelerating detachment of the trailing head ("mechanical gate model")

Impairing this mechanism should prolong the dwell time or increase the number of futile steps (specifically side steps). Thus, both velocity and run length would decrease. If prolonging the dwell time effect is dominant, then only velocity decreases. Alternatively, there may exist some sort of compensation mechanism. For example, positively charged residues (e.g. Lys) would prolong the run length of the molecules.

Thus again only velocity decreases.

1-2) Inhibition of ATP binding to the leading head ("chemical gate model")

Inhibiting this should cause simultaneous detachment or increase the number of futile steps (specifically back steps). Thus, both run length and velocity would decrease. If the simultaneous detachment effect is dominant, then only run length decreases.

2-1) Inhibiting rebinding to the detached position

Lowering internal strain should increase the frequency of binding to the detached head's original position. Experimentally, this would be indicated by a prolonged dwell time in a low temporal resolution study. Consequently, slower velocity and a shorter run length should occur.

2-2) Acceleration of forward binding

Futile steps (either side steps or back steps) should increase causing both velocity and run length to decrease.

From our results, we prefer mechanism 1-2) (chemical gate model) since other mechanisms affect velocity more than run length (in other words, only impairing mechanism 1-2) directly accelerates detachment of the dimer from the MT). Furthermore, since the neck linker is known to dock onto the head following ATP binding, ATP binding can be detected by observing a repositioning of the neck linker. smFRET results showed that both heads from neck linker extended mutants (protein-only Gn mutants; Figure S12C) were in the docked state in saturated AMP-PNP

concentration, something not observed in wild type constructs (Tomishige et al, 2006; Isojima, Toyokita and Tomishige personal communication). Thus, both heads from the mutant construct may be bound to ATP simultaneously at some point during walking. This possibility also applies to DNA-kinesin.

However, these mechanisms are not mutually exclusive. Further study using single molecule techniques (such as FIONA, smFRET and optical-trap) might reveal the contribution of each mechanism. For that, we think kinetic approaches are essential since these can compare each mechanism quantitatively.

2-3. Conclusion

Unique characteristics of DNA-kinesin allowed us to consider the contribution of internal strain on processive movement. Internal strain acts on run length more so than on velocity, meaning that internal strain decreases the likelihood of simultaneous head detachment with minimal effect on ATPase activity. However, these results were obtained from mutants. We hope that in the future, more precise analysis of kinesin activity will elucidate the internal strain mechanism. For such a purpose, more quantitative and kinetic analysis is necessary.

Sub Figure legends

Figure S1. Single molecule imaging of parallel type DNA-kinesin

(A) Structure of parallel type DNA-kinesin. The coiled-coil part of kinesin was replaced with DNA. Since TAMRA and Cy5 were closely located, a high FRET signal was expected to be observed. (B) Anti-correlation of the fluorescence intensity of fluorescent spots attached to an axoneme at 1 mM AMP-PNP. Upon Cy5 photo bleaching (arrow), a TAMRA signal appeared, which then photo bleached in one step (arrow head), indicating a single DNA-kinesin molecule. (C) Histogram of the fluorescence intensity (left) and FRET efficiency (right) of fluorescent spots observed at 1 mM AMP-PNP (upper) or 1 mM ATP (lower). These data suggest that the observed motile fluorescent spots are those of single DNA-kinesin molecules.

Figure S2. Confirming hybridization of anti-parallel type DNA-kinesin.

(A) Structure of anti-parallel type DNA-kinesin in which 10 bp DNA was used. (B) When sense DNA-kinesin and antisense DNA-kinesin were mixed, a FRET signal and motile molecules were observed. (C, D) When only sense-sense or antisense-antisense DNA-kinesins were mixed, no FRET signal and no motile molecules were observed.

Figure S3. FRET signal of anti-parallel type DNA-kinesin suggests a duplex structure for hybridized DNA.

(A) Kymographs of anti-parallel type DNA-kinesin with 6, 10, or 15 bp DNA. Images were obtained with green laser (514 nm) excitation. (B) Fluorescence intensity of DNA-kinesin. (C) FRET efficiency profile of DNA-kinesin. (D) FRET signals can be explained by the duplex structure of DNA, which suggests correct hybridization of the

dsDNA in DNA-kinesin. The solid line is the theoretical curve with a Förster distance of R_0 = 5.5 nm. The theoretical inter-dye distance, *R*, is calculated as a vector sum of components parallel (R_a) and perpendicular (R_b) to the cylinder axis. R_a (= 3.4 N + L) is a function of the number of bp (N) and L = 0.5 nm, which was defined as the distance between the dye for N = 0. $R_b = [a^2 + d^2 - 2adcos(\theta)]^{1/2}$ is a function of a = d = 1.5 nm, the acceptor and donor distances from the axis and the angular separation θ (= 36 N + ϕ), as shown. ϕ = 225° is the inter-dye angle for N = 0. The error bars of the data represent ± SD from the Gaussian function fitting parameter of the main peak from the data in C.

Figure S4. Motile properties of anti-parallel type DNA-kinesin.

Data of the construct connected at positions 333 and 328, which was used in Figure 6 and 7 of the main text. (A) The velocity (left) and run length (right) profile show the dependence on DNA length. (B) To compare, we plotted the relative values against DNA length. Data was normalized using the data from 6 bp (2.4 nm) for position 333 and from 7 bp (2.9nm) for position 328. Run length (blue square), residence time (open triangle; original data was not shown) and motile probability (open diamond; data from Figure 6 of main text) decreased faster than velocity (red circle). To obtain the run length and residence time, data were fitted by nonlinear least squares fitting of the cumulative probability distribution [C1*(1 – exp (-t/C2)) - C3 from t=0 to infinity] where C1 is a normalized parameter and C2 is the run length or the residence time. C3 was used to exclude the effect of the counting loss.

Figure S5. Kymographs and motile probabilities of Mid constructs.

(A) Kymograph of Mid constructs (20 bp DNA) were obtained by simultaneous

excitation by green (514 nm) and red (635 nm) lasers, showing that the Mid constructs do not move processively, but merely by diffusion. (B) Mid constructs do not have any apparent peaks.

Figure S6 Displacement and Mean Square Displacement (MSD) of DNA-kinesin.

(A) Displacements of several DNA-kinesin constructs. The plus end of the axoneme was determined by the movement of wild type dimer kinesin, which is dye-labeled in a different color from DNA-kinesin. Monomer constructs showed no movement. C-terminal (328) and N-terminal (2, 7) connected constructs showed unidirectional movement. Mid connected constructs (23, 101) showed bi-directional movement. Note: some Mid constructs (43, 215) showed little displacement, so these data are not shown. (B) (upper) The labeled positions. (lower) MSD data of some typical constructs. Slope of WT (K490CLM 416) and N-terminal (2) constructs was nearly 2, suggesting that both constructs move with an active transport mechanism. In contrast, the slope of the Mid construct (101) was nearly 1, suggesting that this construct moves by diffusion. The obtained slopes are 2.0, 1.8, 2.0, 1.6, 1.2, and 0.9 for WT (K490CLM 416), 328_7 bp, 2_18 bp, 7_18 bp, 23_20 bp, and 101_20 bp respectively. From the data of (A) and (B), we classified N-terminal constructs 2 and 7 as processive motors and Mid constructs 23, 43, 101 and 215 as diffusional motor constructs.

Figure S7 Displacement and Mean Square Displacement (MSD) of hetero-dimer DNA-kinesin.

(A) Displacements show a forward bias for all constructs except 215-337. These results are expected since one head (position 337) of the hetero-dimer can achieve full bias

through the neck linker. (B) Location of connect positions and MSD data for constructs 324-337 and 23-337. The obtained values were b=1.9, 1.7, 1.5, 1.5 and 1.5 for hetero-dimers 324-337, 23-337, 43-337, 101-337 and 215-337. From the data of (A) and (B), we concluded that hetero-dimeres 324-337 and 23-337 move processively.

Figure S8 Comparison of DNA-kinesin and native kinesin structures.

(A) Structure of native kinesin. The neck linker, which connects the head and coiled-coil of kinesin, acts as an entropy spring, while the coiled-coil acts as a rigid rod. Thus the structure resembles two heads attached to a rod. (B) Structure of DNA-kinesin. The linker (see Figure S9 for details) acts as an entropy spring while the DNA acts as a rigid rod. Thus the structure resembles a rigid rod with a spring connected to both heads. (C) Distribution of one head by fixing the other head at the origin. Distribution of the neck linker-extended construct is shown for native kinesin. The mean end-to-end distance was set to 7 nm for both DNA-kinesin and native kinesin. The distribution of DNA-kinesin shows a sharp contrast with that of native kinesin. (D) Since short dsDNA can be treated as a rod, the area accessible by the detached head is restricted to a doughnut shaped area. Furthermore, the width of the doughnut shaped area is constant for various DNA lengths.

Figure S9 Schematic diagram of the distribution probability of DNA-kinesin

(A) Precise structure of the connection between DNA and kinesin. The linker acts as an entropy spring. (B) For DNA-kinesin, the spring is located at both DNA ends, but for simplification we assumed that the spring exists only at one end. (C) Distribution

probability of DNA-kinesin using eq. S4. See supplementary results for detail.

Figure S10 Kinesin structure and schematic diagram of the collision frequency of the DNA-kinesin detached head.

(A) To calculate the collision frequency, we assumed that kinesin assumes only two structures. One is a docked state, where the neck linker attaches to the head; the other is an undocked state, where the neck linker detaches from the head and moves freely. (B) Recently, another structure (back-docked state) has been reported where the neck linker attaches to the back part of the head. This structure has been hypothesized for the nucleotide-free head, but since this has not yet been obtained for conventional kinesin, this structure was not used in this study. In the two-head bound state, the leading head is thought to keep a nucleotide-free state (ϕ state). Thus, if kinesin takes the back-dock structure in the ϕ state, the distance between the two heads becomes shorter than that used in this study, resulting in a weaker internal strain for the same length of DNA. (C-F) The collision frequency for constructs connected at position 328 are shown. (C) 328 is the mid point of the neck linker. (D) To calculate the distance *D* between two connect positions, we assumed that the size *dr* of the connect position is 0.1 nm. (E) Collision frequencies of many DNA lengths were calculated from the distribution probability. Note: the shape of the distribution does not change for short dsDNA. (F) Calculated collision distribution for the 328 connected construct, assuming that one head, which is the bound head in the one-head bound state and the trailing head in the two-head bound state, is in the docked state. The other head, which is the detached head in the one-head bound state and the leading head in the two-head bound state, is in the undocked state.

Figure S11 Velocity and run length depends on the estimated internal strain.

(A) Parameters for calculating internal strain. See supplementary results for detail. (B) Velocity depends on the estimated internal strain (same as Figure 7B). However, this difference might not be the result of the internal strain but simply the result of the connect position. To exclude this possibility, we used another approach to change the linker length of DNA-kinesin that changes the spring property of DNA-kinesin without changing the mean distance between heads (Figure 7). (C)-(E) show supporting data. (C) Three kinds of carbon chain linkers were used. (D) Collision frequencies are almost the same, which is reasonable since the DNA length defines the collision frequency for DNA-kinesin. With this unique characteristic, we could evaluate the effect of the distance between heads and internal strain independently. (E) Estimated internal strain. The estimated internal strains of AMAS are 1.5-3.0 times larger than those of KMUS.

Figure S12 Evaluating the contribution of the power stroke and communication through the neck linker.

(A) The dual function of the neck linker is essential for processive runs. (B) To evaluate the contribution of the two functions, 4 conditions were compared. Three of them were examined in the main text. Here, the remaining condition (*) was explored. (C) Some protein-only constructs were used. For such a purpose, poly-glycine residues were inserted within the boundary of the neck linker and the coiled-coil. (D) DNA-kinesin with a truncated neck linker was also used.

Figure S13 Models to explain the effect of internal strain.

Internal strain may (A) accelerate detachment of the trailing head; (B) inhibit attachment of ATP to the leading head, thus preventing simultaneous detachment of both heads; (C) block re-attachment of the detached head to the original binding site; or (D) accelerate binding of the detached head to the forward next binding site. Internal strain is indicated by the red arrow. (E) Side or back steps decreased the run length and velocity, which might explain the slow speed and low motile probability of the long DNA condition. Internal strain might regulate these processes.

Table S1 List of oligo DNA.

Sense sequences (labeled with Cy3 or TAMRA) of the used DNA are shown. The KpnI site is underlined. (A) Parallel type. To make a DNA-kinesin dimer, a K20T13 oligo labeled DNA-kinesin monomer was hybridized with a DNA-kinesin monomer labeled with the antisense sequence of K20_S. (B) Anti-parallel type. (C) Control sequence of the anti-parallel type. These sequences gave similar results to (B), but the data were not used in this paper.

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